MBMG
Metabarcoding & Metagenomics

Metabarcoding and Metagenomics 5: 1–15
DOI 10.3897/mbmg.5.57278

Review Article

Application of propylene glycol in DNA-based studies of invertebrates

Alexander M. Weigand[1], **Noah Desquiotz**[2], **Hannah Weigand**[1], **Nikolaus Szucsich**[3]

1 *National Museum of Natural History Luxembourg, Luxembourg, Luxembourg*
2 *University of Luxembourg, Faculty of Science, Technology and Medicine, Esch-Belval, Luxembourg*
3 *Naturhistorisches Museum Wien, Vienna, Austria*

Corresponding author: Alexander M. Weigand (alexander.weigand@mnhn.lu)

Academic editor: Miklós Bálint | **Received** 5 August 2020 | **Accepted** 11 December 2020 | **Published** 4 January 2021

Abstract

High-throughput sequencing (HTS) studies on invertebrates commonly use ethanol as the main sample fixative (upon collection) and preservative (for storage and curation). However, alternative agents exists, which should not be automatically neglected when studies are newly designed. This review provides an overview of the application of propylene glycol (PG) in DNA-based studies of invertebrates, thus to stimulate an evidence-based discussion.

The use of PG in DNA-based studies of invertebrates is still limited (n = 79), but a steady increase has been visible since 2011. Most studies used PG as a fixative for passive trapping (73%) and performed Sanger sequencing (66%; e.g. DNA barcoding). More recently, HTS setups joined the field (11%). Terrestrial Coleoptera (30%) and Diptera (20%) were the most studied groups. Very often, information on the grade of PG used (75%) or storage conditions (duration, temperature) were lacking. This rendered direct comparisons of study results difficult, and highlight the need for further systematic studies on these subjects.

When compared to absolute ethanol, PG can be more widely and cheaply acquired (e.g. as an antifreeze, 13% of studies). It also enables longer trapping intervals, being especially relevant at remote or hard-to-reach places. Shipping of PG-conserved samples is regarded as risk-free and is authorised, pinpointing its potential for larger trapping programs or citizen science projects. Its property to retain flexibility of morphological characters as well as to lead to a reduced shrinkage effect was especially appraised by integrative study designs. Finally, the so far limited application of PG in the context of HTS showed promising results for short read amplicon sequencing and reduced representation methods. Knowledge of the influence of PG fixation and storage for long(er) read HTS setups is currently unavailable.

Given our review results and taking difficulties of direct methodological comparisons into account, future DNA-based studies of invertebrates should on a case-by-case basis critically scrutinise if the application of PG in their anticipated study design can be of benefit.

Key Words

alternative fixative, bioassessment, DNA integrity, DNA preservation, environmental monitoring

Introduction

DNA-based high-throughput sequencing (HTS) approaches such as DNA metabarcoding have lately revolutionised our ability to comparatively assess and monitor biodiversity over large geographical scales and at an unprecedented rate (Taberlet et al. 2012; Aylagas et al. 2016; Leese et al. 2018; Compson et al. 2020). Of particular relevance in practice are declining costs per sample, which are driven, among other things, by laboratory automatisation, comprehensive parallelisation of samples (i.e. multiplexing) and ever decreasing sequencing expenses (Leese et al. 2018). On the other hand, large-scale environmental programmes require a high number of appropriately conserved samples. Assuming that the number of samples to be processed within a scientific study or environmental program will continue to increase, also field costs per sample will account for an increasingly large financial share. It is therefore important to develop strategies to counterbalance the increasing costs of field

Copyright *Alexander M. Weigand et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

PENSOFT

work, e.g. by reducing the time spent in the field, lowering hands-on times per sample by automatisation or adjusting collection and storage conditions (Holderegger et al. 2019; Ssymank et al. 2018).

The fixative (or trapping / killing agent) is a crucial parameter for DNA-based studies as it has to secure the integrity of the DNA from the very beginning (see e.g. Stein et al. 2013). Simultaneously, its costs are directly linked to the number of samples to be processed and to the volumes used. It is not unusual for larger studies to require several hundred to thousand litres of fixative. Liu et al. (2020) identified lab grade ethanol (>95%) to be the fixative of choice in the majority of DNA metabarcoding studies conducted between 2015 and 2019. Yet, lab grade ethanol is very expensive, has a high volatility, is not always easy to obtain and due to its flammability can cause problems during sample transportation and shipping. Alternative fixatives exist but are not widely applied, e.g. due to misunderstandings of chemical properties, tradition or simply lack of knowledge and availability (Weeks Jr and McIntyre 1997; Thomas 2008; Nagy 2010; Stoeckle et al. 2010; Gossner et al. 2016; Mahon et al. 2017).

As such an alternative, propylene glycol (or propane-1,2-diol; here further abbreviated as PG) has a number of characteristics that potentially render it beneficial in large-scale DNA-based HTS bioassessment and biomonitoring programmes: (i) low acquisition cost but high general availability, as it can be bought, for example, as low-budget antifreeze in specialist car dealers or as an additive from the cosmetic or food industry (additive E1520), (ii) non-toxicity (i.e. considered as a GRAS (generally regarded as safe) material), (iii) very low volatility, (iv) environmental safety, (v) risk-free transport of samples according to the regulations of the International Air Transport Association (IATA), and (vi) ability to ensure DNA integrity as well as to preserve most morphological characteristics (Thomas 2008; Nagy 2010).

Propylene glycol is a well-established agent in molecular cryobiology, notably used for the cryopreservation of sperms and cell cultures (Bank and Brockbank 1987; Hezavehei et al. 2018). For the study of invertebrates, it has been proposed as the chemical of choice in a standardised pitfall trap design for monitoring ground-active arthropod biodiversity (Brown and Matthews 2016; Hohbein and Conway 2018) and is applied as such within the North American National Ecological Observatory Network (NEON; Gibson et al. 2012; Hoekman et al. 2017) and the carabidologist community (Kotze et al. 2011). PG-based pitfall traps were also proposed as a standard and minimally disturbing method to investigate the subterranean fauna of the mesovoid shallow substratum (López and Oromí 2010; emptied every six months). Since 2001, PG fixation is applied by the Soybean Aphid Suction Trap Network (STN; Lagos-Kutz et al. 2020) for monitoring the 'aerobiological soup'. In the context of environmental genomic studies, Matos-Maraví et al. (2019) proposed PG as the fixative of choice for mass samples originating from pitfall traps and flight interceptions, but neither tests nor metadata had been published along with this statement.

The aim of this review is to summarise the findings of studies investigating the application of PG for DNA-based analyses of invertebrates. We will distinguish its application as a fixative (during sample collection) and as a preservative (for sample storage and curation). The collated information should help to transfer the available knowledge to the wider community, stimulating an evidence-based discussion on how to further reduce costs for DNA-conform sample collection and curation in larger environmental programmes by exploring alternative fixation and preservation agents.

Material and methods

Literature search

A topical core literature research was conducted on 07.10.2020 within the ISI Web of Science (WoS) and screening 'all databases'. The following search strings were investigated: (1) "propylene glycol" AND "DNA" AND "invertebrate*", (2) "propylene glycol" AND "DNA" AND "insect", (3) "propane-1,2-diol" AND "DNA" AND "invertebrate*" and (4) "propane-1,2-diol" AND "DNA" AND "insect". On the very same day, and because the WoS search only will detect literature records which are ISI-listed and only will retrieve hits in case the search string terms appear in the title, abstract, topic or as keywords, a complementing Google Scholar (GS) search was performed (as e.g. proposed by Piasecki et al. 2018). The GS search enables in-text searches and will also retrieve grey literature records such as field, laboratory and shipping protocols, pre-prints, theses and agency reports. The same four search strings were entered, selecting the 'exclude patents' option in GS. The keyword 'sperm*' was excluded in all searches as PG is frequently applied during the cryopreservation of sperms which would have resulted in a very high number of irrelevant hits. The key word 'invertebrate*' was excluded in the search strings (2) and (4) to not again screen insect literature records retrieved from (1) and (3).

The four search strings yielded the following number of literature records within the ISI WoS: (1) 20, (2) 8, (3) 0 and (4) 1. From those, only records which transparently stated the application of PG during specimen handling have been considered, resulting in a total of only 12 studies. Google Scholar search strings yielded the following constant number of literature records, regardless of whether the search was performed via two different IP-addresses in two countries (Luxembourg and Germany), within two different Google profiles, using a private browser window or being logged on/off from personal Google profiles: (1) 509, (2) 1930, (3) 23 and (4) 432.

Results

The majority of studies had to be excluded, a) because they used PG as a trapping material for invertebrates but only

cited literature referring to the application of DNA-based tools, or b) as they referred to yeast species molecularly analysed. Furthermore, reviews or studies which only dealt with the DNA analysis of e.g. parasites or gut contents of invertebrates were omitted, but will be discussed. Doctoral and master theses which were subsequently published as scientific articles were counted as a single entry.

A total of 79 publications was retrieved (Table 1, Fig. 1). Although the restricted number of literature records obtained exemplifies that the number of studies using PG as a fixative or preservative for subsequent molecular DNA-based analysis of invertebrates is not widely established (but an increase can be noted), still some patterns can be inferred. The analysed studies almost exclusively investigated terrestrial taxa, with Robinson et al. (2020; freshwater bulk sample) and Cordero et al. (2017; aquatic insects) being notable exceptions. The first studies using PG for DNA-based analysis of invertebrates originate from 2003 and 2005. Two of them focussed on honey bees (Rubink et al. 2003; Coulson et al. 2005) while Carter (2003) performed a comprehensive study on '*The effects of preservation and conservation treatments on the DNA of museum invertebrate fluid preserved collections*' using a terrestrial isopod species as reference. Those studies were accompanied by early publications on arachnids (Vink et al. 2005) and hemipterans (Scott et al. 2007). From today's perspective, studies focussing on Coleoptera (n = 24 studies, 30%) and Diptera (20%) dominated the dataset (Fig. 1). Although Diptera was the second largest of the individual groups retrieved, the first study was only published in 2012 and most of the records originate from the same team of authors.

In the majority of study designs, PG was used as a fixative for passive trapping (n = 53, 73%), and less frequently as a fixative upon manual collection of living specimens (32%). Either food-/laboratory-grade PG (14%; Sigma-Aldrich, Ajax FineChem, Neogen, Herrlan-PSM, Old World Industries, Better World Manufacturing, ClassiKool Ltd.) or PG-based antifreezes (13%; Lowtox, Absolute Zëro RV Waterline, Sierra, Uni-Gard) were used, but in 75% of all studies no further chemical properties or customer specifications were provided. PG concentrations were in most cases higher than 95% (for 70% of study designs), in fewer instances between 50–75% (16% study designs) or below 50% (11% study designs). Information on storage conditions for PG-preserved specimens was also very scarce: 27% of studies did not report any storage duration and for 41% of studies no storage temperatures were provided. Otherwise, PG-preserved specimens were stored for quite variable time spans, i.e. for more than half a year (10%), 1–6 months (10%), 1–4 weeks (20%), below 1 week (17%) or even for shipping only (17%, variable duration). If information was provided, specimens most often were stored at RT (28%), less frequently frozen (17%) or refrigerated (10%). Sanger sequencing was the most frequent evaluation method (66%), followed by PCR-based analyses (16%), HTS (11%) and microsatellite genotyping (9%). Studies performing two conceptual approaches, e.g. Sanger sequencing of COI and COI metabarcoding, were included in both categories.

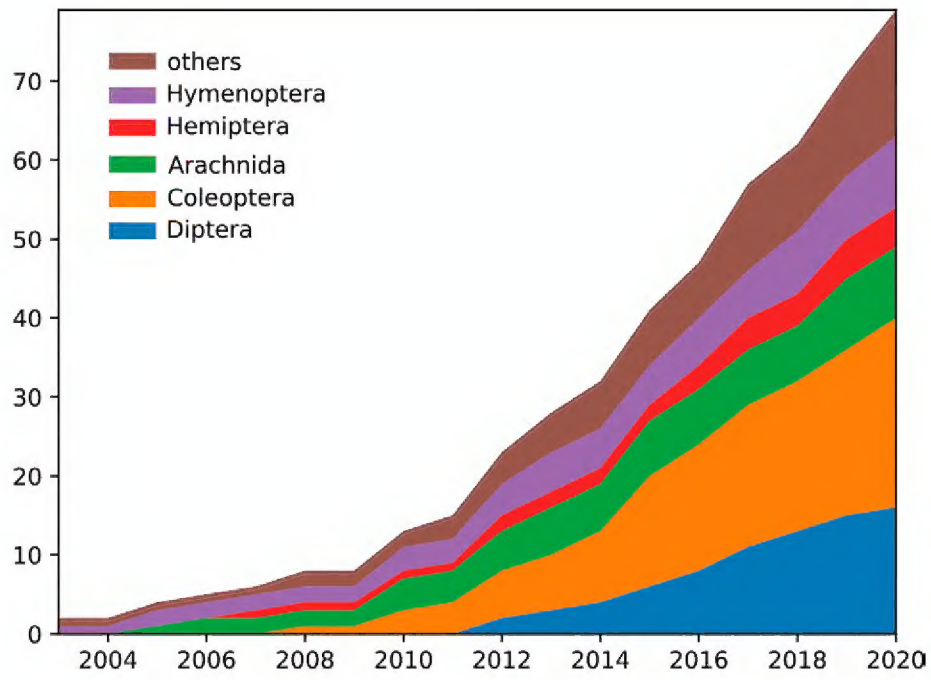

Figure 1. Cumulated number of studies which have used propylene glycol either as a fixative or preservative in the DNA-based analysis of invertebrates. Arranged by the taxonomic groups in focus. In cases where several of the indicated groups were targeted, the study was placed into the category "others". N = 79, as of 07.10.2020.

Discussion

Application of propylene glycol as a fixative

Our results indicate that PG is widely applied as a fixative in a variety of passive trapping methods (e.g. pan trap, funnel trap, aerial pitfall trap; baited and unbaited) and for various organism groups (mainly beetles and flies, but also spiders, bees and aphids). Likewise, actively collected single specimens or – in a few cases – invertebrate bulk samples were fixed with PG (e.g. Bowser et al. 2017, 2019; Cordero et al. 2017; Jusino et al. 2019; Robinson et al. 2020; Liu et al. 2020). This taxonomically widespread application mimics the use of PG in traditional non-DNA-based research and application domains (primarily pitfall trapping; see Hohbein and Conway 2018) now entering the molecular field. Research communities around aphids, spiders, carabids and tephritid fruit flies contributed most of the studies.

Amongst others, passive trapping intervals are determined by the accessibility of the sampling location, the volume of the trapping containers, the evaporation rate of the fixative and local environmental parameters such as humidity, temperature, rainfall or UV-exposure. In case of excessive heat and high temperatures (arid and hot), traps with ethanol or (salted) water frequently dry out and either have to be visited and re-filled more frequently, or short(er) trapping periods have to be chosen. The evaporation rate of PG is >500-times lower than for ethanol (Moreau et al. 2013). As such a low volatile agent, PG-equipped traps can retain their volumes more or less constant over several weeks to months (Hohbein and Conway 2018). However, long-term UV exposition may decompose PG into water, acetone and 2-propanol (i.e. isopropanol) (Nakahama et al. 2019). In interaction with its hygroscopic nature, even apparently large fixative volumes at the end of a long trapping period under summer conditions might contain a

Table 1. Overview of studies which have used propylene glycol either as a fixative or preservative for the DNA-based analysis of invertebrates. RT = room temperature; PG = propylene glycol; n.a. = not applicable or not available. Studies found by both the ISI Web of Science and Google Scholar searches, are marked in bold. All others were only detected by the latter search engine. N = 79, as of 07.10.2020.

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Carter (2003)	2003	Crustacea (Isopoda)	PCR-based visualisation (16S, 18S)	Propylene glycol	specimens directly placed in pure PG	12 months (RT)			double-stranded DNA profiles of PG-conserved specimens were sufficient for PCR, but likely not adequate for long-term storage of museum samples
Rubink et al. (2003)	2003	Hymenoptera (Apidae)	Microsatellite and CytB fragment analyses	Low-toxicity antifreeze (Lowtox, Prestone Inc., Danbury, CT)	specimens directly placed in pure PG	5, 20 or 90 days (20 °C or 40 °C)	95% ethanol	up to 4 months (4–6 °C)	nuclear and mtDNA were amplifiable even at the most extreme conditions (90 days, 40 °C), although a slightly decreasing trend was observed
					aerial pitfall trap with PG	3 weeks			
Coulson et al. (2005)	2005	Hymenoptera (Apidae)	CytB, 16S and COI fragment analysis	Propylene glycol	baited aerial pitfall trap with 50% PG and soap	ca. weekly for 8 months	95% ethanol	n.a.	well preserved for molecular analysis
Vink et al. (2005)	2005	Arachnida (Aranea, Scorpiones)	Actin and COI fragment analysis	Propylene glycol (99.5+% laboratory grade, Sigma-Aldrich)	specimens directly placed in pure PG	6 weeks (40 °C, 19–24 °C, 2–4 °C, -20 °C or -40 °C; dark)	95% ethanol (4 °C)	1 day	PG (and RNAlater) significantly better preserved nuclear and mtDNA than ethanol at various concentrations and in different study designs: mtDNA successfully amplified under most extreme conditions (6 weeks, 40 °C); ncDNA for small soft-bodied species only at room temperature or lower, and for large heavily sclerotized species at 2–4 °C or lower
Hendrixson (2006)	2006	Arachnida (Aranea)	Sanger sequencing of COI and 28S	Propylene glycol	pan trap with 1:1 PG and 100% ethanol	each 2 weeks for 2 months	100% ethanol, than 80% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Scott et al. (2007)	2007	Hemiptera (Aleyrodidae)	Sanger sequencing of COI	Propylene glycol	specimens directly placed in pure PG	n.a.			well preserved for morphological and molecular analyses
Gallego and Galián (2008)	2008	Coleoptera (Curculionidae)	Sanger sequencing of COI	Propylene glycol	funnel trap with pure PG	1 week	absolute ethanol	n.a.	well preserved for morphological and molecular analyses, in particular in periods of no rainfall
Villacorta et al. (2008)	2008	Crustacea (Amphipoda)	Sanger sequencing of COI, COII and H3	Propylene glycol	baited pitfall trap with PG	several weeks to months	ethanol	n.a.	well preserved for morphological and molecular analyses
Castalanelli et al. (2010)	2010	Coleoptera	Sanger sequencing of mtDNA, single and multi-copy ncDNA genes	Propylene glycol	specimens directly placed in 20% PG	n.a.	ethanol	n.a.	very fast DNA isolation (ranging from 2–20 minutes); well preserved for parallel morphological and genetic analyses; specimens stored in 20% PG and ethanol did not differ from specimens stored in other preservatives
Horn (2010)	2010	Hymenoptera (Anthophila)	Sanger sequencing of COI	Propylene glycol	pan trap with 75% PG	each two weeks	95% ethanol (refrigerator)	n.a.	specimens suitable for barcoding
Malumbres Olarte (2010)	2010	Arachnida (Aranea)	Sanger sequencing of COI	Mono-propylene glycol	pitfall trap with pure PG	2 weeks over 3 months	95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Shoda-Kagaya et al. (2010)	2010	Coleoptera (Curculionidae)	Microsatellite genotyping	Propylene glycol	pheromone-baited trap with PG	n.a.	99.5% ethanol (-20 or 4 °C)	n.a.	specimens suitable for microsatellite analysis
Sonoda et al. (2010)	2010	Arachnida (Aranea)	Sanger sequencing of COI and restriction site analysis	Propylene glycol	pitfall trap with 20% PG	1 or 2 weeks	70% ethanol	months	well preserved for morphological and molecular analyses
Boyer et al. (2011)	2011	Annelida (Oligochaeta)	Sanger sequencing of COI and 16S	Propylene glycol	specimens directly placed in 98% ethanol	n.a.	PG	n.a.	well preserved for morphological and molecular analyses
Stevens et al. (2011)	2011	Coleoptera (Tenebrionidae, Bostrichidae)	Arginine kinase fragment analysis	99.5% Propylene glycol (Sigma-Aldrich Inc, St. Louis, MO)	specimens directly placed in PG (100%, 80%, 50% diluted or with PBS, with / without Triton-X 100)	3, 7 or 14 days (30 °C)	absolute ethanol (Sigma-Aldrich, molecular grade) (-80 °C)	n.a.	treatments with specimens stored in mixtures containing PG produced significantly less successful PCR results. PCR success was higher for specimens stored in pure PG than for 80% PG
				99.7% propylene glycol (Ajax FineChem Pty Ltd, Taren Point NSW, Australia)	Lindgren funnel trap with PG	1 week			specimens in PG produced significantly less successful PCR results than specimens maintained in PBS or dry
Castalanelli et al. (2012)	2012	Coleoptera (Dermestidae)	Sanger sequencing of COI, CytB and 18S	Propylene glycol	baited lure trap with 20% PG	two months	rinsed with sterile water, rinsed with 70% ethanol and stored in 95% ethanol	n.a. (-20 °C)	well preserved for morphological and molecular analyses

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Gibson et al. (2012)	2012	Coleoptera (Carabidae)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 66% PG	1 week	75% ethanol, or rinsed in water and stored in 95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Gruber et al. (2012)	2012	Hymenoptera (Formicidae)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 33% PG	1 day	95% ethanol (4 °C)	n.a.	well preserved for morphological and molecular analyses
Knee et al. (2012), Knee (2012)	2012	Arachnida (Acari)	Sanger sequencing of COI and 28S	Propylene glycol	baited Lindgren funnel trap with PG	~2 weeks	95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Pelletier et al. (2012)	2012	Hemiptera (Aphidae)	Sanger sequencing of COI	Propylene glycol	trap with 50% PG with Bitrex and soap	each 2–3 days for 2 months	50% PG (4 °C)	up to 1 week	PG concentration checked in the field remained in the range 40–60%; collected material mostly suitable for morphological analysis, barcoding and RNA virus detection; although higher concentrations yielded better results
Renaud et al. (2012), Renaud (2012)	2012	Diptera (Muscidae)	Sanger sequencing of COI	Propylene glycol (food quality)	pan trap with 33% PG and soap	3–4 days	n.a.	n.a.	handling of PG was problematic because treated as hazardous waste and forbidden to dispose in local septic system; specimens suitable for barcoding and morphological analysis
Schutze et al. (2012)	2012	Diptera (Calliphoridae, Fanniidae, Muscidae, Tephritidae)	Sanger sequencing of COI	Propylene glycol	lure-baited hanging trap with pure PG	n.a.	assumably pure PG	n.a.	easy transport of samples; >90% of specimens morphologically characterised and barcoded
Vélez et al. (2012)	2012	Myriapoda (Chilopoda)	Sanger sequencing of COI and 16S	Propylene glycol	specimens directly placed in 95% or 75–80% ethanol	n.a.	PG, than 96% ethanol	n.a.	effective shipping of samples; well preserved for molecular analysis
Ferro and Park (2013)	2013	Coleoptera (Carabidae, Staphylinidae)	Sanger sequencing of COI	Propylene glycol (Neogen Corporation, Item No. 79231),	specimens directly placed in 100% ethanol	2 days	20%, 40%, 60%, 80% and pure PG (21 °C)	up to 6 months	positive PCR or sequencing results were obtained in all cases except for 20% PG
Krosch et al. (2013)	2013	Diptera (Tephritidae)	Microsatellite genotyping	Propylene glycol	insecticide-baited hanging trap with PG	n.a.			effective shipping of samples; well preserved for morphological and molecular analyses
Moreau et al. (2013)	2013	Hymenoptera (Formicidae)	Long-wavelength rhodopsin and COI fragment analysis	100% food-grade PG	specimens directly placed in pure PG	either remaining in PG for up to 10 months, or transferred into 95% ethanol after 6 months (6+4 months storage time)			PG and ethanol allowed for the highest PCR success rates. PG-preserved samples showed comparatively high DNA concentrations even after 10 months
Sikes and Stockbridge (2013), Stockbridge (2013)	2013	Mecoptera	Sanger sequencing of COII	Propylene glycol based antifreeze (Sierra brand)	pitfall trap with PG	two weeks	100% ethanol (-70F)	n.a.	well preserved for molecular analysis
Sim (2013)	2013	Arachnida (Aranea)	Sanger sequencing of COI, ITS and ND1	Propylene glycol	pitfall and pan trap with 50% PG	3–4 days for 2 weeks	95% ethanol (4 °C)	n.a.	well preserved for morphological and molecular analyses
Boykin et al. (2014)	2014	Diptera (Tephritidae)	Sanger sequencing of COI, NAD4–3', CAD, period, ITS1 and ITS2	Propylene glycol	insecticide-baited hanging trap with PG	variable	100% ethanol	n.a.	effective shipping of samples; well preserved for morphological and molecular analyses
Endo et al. 2014	2014	Coleoptera (Carabidae); Myriapoda; Collembola	Sanger sequencing of COI, ITS1 and ANT	Propylene glycol	pitfall trap with PG:ethanol (1:1)	n.a.	100% molecular-grade ethanol	n.a.	well preserved for molecular analysis
Gómez (2014)	2014	Coleoptera (Carabidae)	Sanger sequencing of COI, CAD and 28S	Propylene glycol	pan trap with PG	n.a.			suitable for all molecular investigations; however, higher amplification success when PG-fixed specimens were dry pinned or transferred to 95% ethanol within 1–2 weeks
Chinvinijkul et al. (2015)	2015	Diptera (Tephritidae)	Sanger sequencing of ITS1	Propylene glycol	specimens directly placed in 95% ethanol	n.a.	pure PG	n.a.	effective shipping of samples; well preserved for molecular analysis
Fountain et al. (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI, CytB and ITS2	Propylene glycol	tarsal clips directly placed in PG	n.a.	95% ethanol (-20 °C)	n.a.	well preserved for molecular analysis
Haase and Zielske (2015)	2015	Gastropoda (Caenogastropoda)	Sanger sequencing of COI, 16S and ITS2	Propylene glycol	specimens directly placed in 70% ethanol	n.a.	PG, than 96% ethanol	PG-preservation only for shipping	effective shipping of samples; well preserved for morphological and molecular analyses
Höfer et al. (2015)	2015	Arachnida (Aranea)	Sanger sequencing of COI	Propylene glycol (technical grade, Herrlan-PSM)	specimens directly placed in pure, 90% or 50% PG	1, 2 and 4 weeks (refrigerator)	non-denatured 96% ethanol	n.a.	succesful barcoding under all conditions, but results potentially indicate a negative effect of water intrusion on PG-preserved specimens

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
					pitfall trap with PG, 2.5% acetic acid and detergent	2 weeks over 6 weeks	70–80% ethanol	n.a.	traps with PG captured more species (at three sites and for the most abundant families), but PG was not selectively attractive for particular taxa
Leblanc et al. (2015)	2015	Diptera (Tephritidae)	Sanger sequencing of COI	Propylene glycol (Better World Manufacturing, Fresno, CA)	baited Lindgren funnel trap with 25% PG	3–5 days	95% ethanol (freezer)	n.a.	well preserved for morphological and molecular analyses
Rugman-Jones et al. (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI and 28S	Pink marine or recreational vehicle antifreeze (not for automobiles)	pheromone-baited Lindgren funnel trap with PG	weekly or bi-weekly	100% ethanol	n.a.	well preserved for molecular analysis; part of specimens collected in PG-based antifreeze according to official monitoring guidelines (Seybold et al. 2013)
Sánchez García et al. (2015), Sánchez García (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI and COII	Propylene glycol	ethanol-baited funnal trap with PG	n.a.	n.a.	n.a.	well preserved for morphological and molecular analyses
Smith and Cognato (2015)	2015	Coleoptera (Curculionidae)	Sanger sequencing of COI and 28S	Propylene glycol	Lindgren funnel trap with PG	n.a.	100% ethanol	n.a.	well preserved for morphological and molecular analyses
Steininger et al. (2015)	2015	Coleoptera (Curculionidae)	Arginine kinase fragment analysis	99% extra pure PG (Fisher) and low-toxicity antifreeze (Lowtox, Prestone Inc.)	specimens directly placed in pure PG or PG-based antifreeze	2 or 7 days			very high qPCR success rates, no matter which storage conditions were used
Borges et al. (2016)	2016	Coleoptera (Zopheridae)	Sanger sequencing of COI, COII, tRNA-Leu gene and EF1α	Propylene glycol	pitfall trap with pure PG	n.a.	100% ethanol or acetone (refrigerator)	n.a.	well preserved for morphological and molecular analyses
Eigenbrode et al. (2016)	2016	Hemiptera (Aphidae)	Microsatellite genotyping	Propylene glycol	pan trap with PG	2-times a week for few months	95% ethanol	n.a.	well preserved for microsatellite analysis
Liu (2016)	2016	Diptera (Drosophilidae)	Microsatellite genotyping	100% food-grade propylene glycol	baited bottle trap with PG	1–2 days	95% ethanol (-60 °C)	n.a.	well preserved for morphological and microsatellite analyses
Patrick et al. (2016)	2016	Diptera (Calliphoridae, Fanniidae, Muscidae, Tephritidae)	High-quality genomic DNA for HTS approaches	99.5% propylene glycol (Sigma-Aldrich)	specimens directly placed in pure PG	1, 8, 13, 14 or 15 days (4 °C or -20 °C)			PG, 97–100% ethanol and AL buffer yielded high-quality genomic DNA, whereas RNA-free water, buffer AE and PBS failed. DNA concentration in ethanol was significantly higher at both temperatures
					lure-baited modified Steiner trap with PG	daily over few weeks	living specimens refrigerated than stored in pure PG (chilled)	20 days, afterwards for max. 1 month (-80 °C)	PG storage was chosen due to its higher overall practicability compared to ethanol and RNAlater; specimens were easily transported in airplane and provided high-genomic DNA for subsequent analyses
Postlethwaite (2016)	2016	Hymenoptera (Anthophila)	Sanger sequencing of COI	Propylene glycol	pitfall trap with pure PG	days to few months	PG until pinning	n.a.	effective shipping of samples; well preserved for morphological and molecular analyses
Robideau et al. (2016)	2016	Coleoptera (Scolytidae)	Real-time PCR of COI	Propylene glycol	Lindgren funnel trap with PG	n.a.	95% ethanol	n.a.	well preserved for morphological and molecular analyses
Wiseman et al. (2016)	2016	Coleoptera (Carabidae)	Sanger sequencing of COI, ITS2, 18S and 28S	Propylene glycol	pitfall trap with PG	n.a.	pinned and dried	n.a.	well preserved for molecular analysis
Boontop et al. (2017a), Boontop (2017)	2017	Diptera (Tephritidae)	Sanger sequencing of COI and microsatellite genotyping	Propylene glycol	specimens directly placed in pure PG	until shipping (RT)	95% ethanol (-20 °C)	n.a.	well preserved for morphological and molecular analyses
Boontop et al. (2017b), Boontop (2017)	2017	Diptera (Tephritidae)	Sanger sequencing of COI and microsatellite genotyping	Propylene glycol	specimens directly placed in pure PG	until shipping (RT)	95% ethanol (-20 °C)	n.a.	effective shipping of samples; well preserved for morphological and molecular analyses
Bowser et al. (2017)	2017	Arthropod sweep net bulk sample	COI metabarcoding	Propylene glycol antifreeze (Uni-Gard -100)	specimens directly placed in pure PG	n.a. (-23 °C)	100% ethanol, rinsed with PBS prior to extraction	21 days	effective shipping of samples; well preserved for morphological and genomic analyses
Cordero et al. (2017)	2017	aquatic insects	Sanger sequencing of COI	Propylene glycol	specimens directly placed in 80% PG	n.a.	95% ethanol	n.a.	specimens suitable for barcoding
Greenslade et al. (2017)	2017	Collembola (Dicyrtomidae)	Sanger sequencing of COI	Mono-propylene glycol (antifreeze)	baited pitfall trap with PG and soap	2 days	Nesbitt solution	over night	well preserved for morphological and molecular analyses
Hoekman et al. (2017)	2017	Coleoptera (Carabidae)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 50% PG	each 2 weeks over entire growing season	95% ethanol, renewed after 24 h	up to several months	specimens highly suitable for morphological and genetic identification

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Langer et al. (2017)	2017	Diptera (Calliphoridae)	Sanger sequencing of COI	nontoxic recreational vehicle antifreeze	baited bottle trap with PG	n.a.	80% ethanol	n.a.	well preserved for morphological and molecular analyses
Lefort et al. (2017)	2017	Hemiptera (Aphidae)	COI metabarcoding	Propylene glycol	specimens directly placed in pure PG	n.a.	n.a. (-80 °C)	n.a.	effective transport of samples; DNA suitable for high-throughput sequencing applications
Perry et al. (2017)	2017	Lepidoptera (Plutellidae)	SNP assay (RAD-Seq)	Propylene glycol (USP grade)	specimens directly placed in pure PG	n.a. (-20 °C)			DNA suitable for population genomic SNP analysis
Ulyshen et al. (2017)	2017	Coleoptera (Lucanidae)	Sanger sequencing of COI	Propylene glycol	baited flight intercept trap with PG	each 2 weeks over 6 months	n.a.	n.a.	specimens well preserved for barcoding
Daglish et al. (2018)	2018	Coleoptera (Bostrichidae)	PCR-based resistance marker screening (*rph2*)	Propylene glycol	lure-baited Lindgren funnel trap with PG	n.a.	n.a.	n.a.	genomic DNA suitable for mass screening of resistance marker
Grando et al. (2018)	2018	Hymenoptera (Anthophila)	Sanger sequencing of COI	Propylene glycol	Vane trap with PG	n.a.	70% ethanol	few months (10 °C)	well preserved for morphological and molecular analyses
Gregoire Taillefer and Wheeler (2018)	2018	Diptera	Sanger sequencing of COI	Propylene glycol	pan trap with 50% PG and soap	7–8 days	95% ethanol or air-dried or ethyl acetate or hexamethyl-disilazane	n.a.	well preserved for morphological and molecular analyses
Ide et al. (2018)	2018	Hymenoptera (Formicidae)	LAMP (loop-mediated isothermal amplification) assay	Propylene glycol	baited pan trap with pure PG	3 h	washed in 99.5% ethanol, air-dried (RT)	n.a.	well preserved for molecular analysis
Muturi et al. (2018)	2018	Diptera (Culicidae; gut content)	Sanger sequencing of COI and 16S metabarcoding	Propylene glycol	suction trap with 50% PG	weekly from May to October	95% ethanol (-20 °C)	n.a.	specimens well preserved for barcoding and microbial gut content analysis
Angelella et al. (2019)	2019	Hemiptera (Aphidae)	SNP assay	Propylene glycol	pitfall trap with 25% PG	weekly for 14 weeks	undiluted ethanol (-80 °C)	n.a.	DNA suitable for population genomic SNP analysis
Bowser et al. (2019)	2019	Arthropod sweep net bulk sample	COI metabarcoding	Propylene glycol antifreeze (Uni-Gard -100)	specimens directly placed in pure PG	n.a. (-23 °C)	100% ethanol, rinsed with PBS prior to extraction	1 week	well preserved for genomic analysis
DiGirolomo et al. (2019)	2019	Coleoptera (Buprestidae)	Sanger sequencing of COI	Propylene glycol	barrel with baited collection cups with PG	several months	n.a.	n.a.	specimens well preserved for morphological analysis, but barcoding was only partly successfull
Jusino et al. (2019)	2019	Arthropod pitfall trap bulk sample	COI metabarcoding	Propylene glycol	pitfall trap with pure PG	2–3 days	100% ethanol (RT, -20 °C)	n.a.	well preserved for morphological and genomic analyses
Krosch et al. (2019)	2019	Diptera (Tephritidae)	Sanger sequencing of COI and COII	Propylene glycol	specimens directly placed in pure PG	n.a. (RT)			effective shipping of samples; well preserved for morphological and molecular analyses
Landi et al. (2019)	2019	Coleoptera (Curculionidae)	Sanger sequencing of COI	Propylene glycol	ethanol-baited Lindgren funnel trap with PG	n.a. (Nov-Feb)	n.a.	n.a.	well preserved for morphological and molecular analyses
Lienhard and Schäffer (2019)	2019	Arachnida (Acari)	PCR-based visualisation	Propylene glycol	specimens directly placed in pure PG	several weeks	absolute ethanol	1 day	results for DNA quality and quantity of PG-conserved specimens similar to ethanol-conserved specimens
Nakahama et al. (2019)	2019	Orthoptera (Gryllidae)	PCR-based visualisation (COI)	Propylene glycol	exposure to ethyl acetate vapour, followed by dehydration in 99.5% ethanol	1 h and 24 h	99% PG	1, 6 and 12 months	all replicates with PG-preserved (n = 12) were successfully amplified for all timepoints and three different fragment sizes
					-30 °C and dehydration in 99.5% ethanol	both 24 h			
					-30 °C	24 h			
Ramírez et al. (2019)	2019	Arachnida (Aranea)	Sanger sequencing of COI, 12S, 16S, H3, 18S and 28S	Propylene glycol (Sierra antifreeze)	pitfall trap with PG	~30 days	95% ethanol for transport; 100% ethanol storage	n.a.	well preserved for morphological and molecular analyses, although heavy rainfall diluted PG in traps and has led to sediment wash-in
Taillefer and Wheeler (2019)	2019	Diptera (diverse Schizophora families)	Sanger sequencing of COI	Propylene glycol	pitfall trap with 50% PG, drop of detergent	6–8 days	95% ethanol	n.a.	well preserved for morphological and molecular analyses
Ballare et al. (2020)	2020	Hymenoptera (Anthophila)	ddRAD sequencing	Propylene glycol	Vane trap with PG, amongst other treatments (e.g. pan trapping with soapy water; netted specimens placed in ethanol)	5 days	100% ethanol	n.a. (until pinning)	all treatments produced a large number of high-quality loci (>4,000, ~20×). In comparison, the two PG-preserved species showed average DNA concentrations, but higher than average mean locus depths and lower than average mean numbers of polymorphic loci

Study	Year	Taxon	DNA-based approach	PG specificities	Fixation step		Preservation step		Central outcomes
					Condition(s)	Duration	Condition(s)	Duration	
Krosch et al. (2020)	2020	Diptera (Tephritidae)	Sanger sequencing of COI	Propylene glycol	specimens directly placed in pure PG	n.a. (RT)			effective shipping of samples; suitable for DNA barcoding
Liu et al. (2020)	2020	Coleoptera (diverse)	COI and 16S metabarcoding	100% food-grade propylene glycol	pitfall trap with pure PG	30 days	95% ethanol	n.a. (-20 °C)	well preserved for morphological and molecular analyses
Mitchell et al. (2020)	2020	Coleoptera (Scarabaeidae)	Sanger sequencing of COI	Propylene glycol	flight intercept trap with PG:water mix	n.a.	ethanol (after 2–4 weeks)	n.a.	well preserved for morphological and molecular analyses
Moricca et al. (2020)	2020	Coleoptera (Curculionidae)	Sanger sequencing of COI	Propylene glycol	lured Lindgren funnel trap with 30% PG	15 days	96% ethanol	n.a.	well preserved for morphological and molecular analyses, as well as for molecular screening of phytopathogenic fungi
Nakamura et al. (2020)	2020	Hymenoptera, Diptera, Coleoptera	PCR-based visualisation (COI)	Propylene glycol	specimens directly placed in 98% PG	2 weeks to 205/215 days (RT)			all, respectively, 96% of PG-preserved specimens produced PCR bands after 2 weeks and 6 months for two primer pairs and the DNeasy Blood and Tissue kit; performance was better than for 99.5% ethanol; PrepMan Ultra kit performance was less sophisticated
Robinson et al. (2020)	2020	benthic macroinvertebrate bulk sample	COI metabarcoding	Absolute Zëro RV Waterline Antifreeze (Recochem, Montreal, QC)	PG added to homogenized mock communities; or samples fixed in PG and than homogenized (ratio 1:3 fixative to sample)	3 days (RT)			evaporation step prior to DNA extraction was ommitted as PG does not inhibit PCR; communities are highly similar and even showed higher proportion of arthropod reads and higher richness than samples conserved with >99% lab grade ethanol

not to be underestimated amount of water and other compounds (Nagy 2010; Goetze and Jungbluth 2013; Borges et al. 2016), but chemical interactions and their effects on medium- to long-term DNA integrity are largely unknown. In addition to high temperatures, water dilution of PG in principle leads to a generally lower DNA integrity (Stevens et al. 2011; Höfer et al. 2015; Patrick et al. 2016; Nakamura et al. 2020), since water can lead to the hydrolysis of nucleic acids. Since most reviewed studies did not indicate the nature of the propylene glycol used, or field and storage temperatures, it was difficult to compare study outcomes. Nevertheless, in several studies, PG was used in the range of 20–80% as a trapping agent and traps deployed for a duration of 1–2 weeks. Specimens collected and fixed under these conditions were still sufficiently preserved for DNA analysis (Coulson et al. 2005; Sonoda et al. 2010; Hoekman et al. 2017; Angelella et al. 2019). In one of the most extreme cases, samples were fixed for 1 month in pitfall traps containing PG-antifreeze. The traps further experienced heavy rainfall and sediment wash-in. Nevertheless, Ramírez et al. (2019) successfully amplified six marker genes for the spider family under investigation and were able to morphologically investigate the specimens at hand.

Propylene glycol is in accordance with the Dangerous Goods Regulations of The International Air Transport Association (IATA). This means that PG-fixed samples are suitable for direct shipping and do not have to be transferred to another chemical agent on the spot. This characteristic was especially important for studies in remote areas (e.g. Schutze et al. 2012; Haase and Zielske 2015; Patrick et al. 2016; Bagnall 2016; Boontop et al. 2017a). In addition, it was seen as advantageous that PG can be relatively easily and cheaply obtained as an antifreeze in many parts of the world – a fact that does not always apply to absolute ethanol. As a major drawback, however, PG-based antifreezes might be regarded as special waste. As such, it can be prohibited to introduce them into local septic systems and the natural ground or to dispose them as domestic waste (Thomas 2008; Renaud 2012). Some national programmes might even prohibit the general use of antifreezes, as they can come along with additives such as lubricants, buffers, corrosion inhibitors and anti-foaming agents, whose impacts on natural environments are often not totally understood – or are considered carcinogenic (e.g. Tolytriazole, see Thomas 2008). Yet, much of the concern and regional bans relate to ethylene glycol-based antifreezes, which are toxic to humans and impose environmental risks. Readily available food-grade PG-based formulations such as from swimming pool or recreational vehicle antifreezes are generally regarded as safe (GRAS) material. They metabolize to lactic acid or substances of the Krebs cycle, which are natural metabolic products in the environment (Thomas 2008; Skvarla et al. 2014). Nevertheless, the waste-disposal issue imposed by using antifreeze fixatives (as it is true for larger quantities of ethanol as well) should be understood beforehand and appropriate measures taken.

Another relevant aspect refers to a potential catch bias caused by the fixative. Although Schmidt et al. (2006) have not integrated PG in their test, they showed that capture efficiencies of commonly used fixatives in pitfall trapping of spiders and carabids can greatly vary. Adding to this, Weeks Jr & McIntyre (1997), Calixto et al. (2007) and McCravy and Willand (2007) demonstrated that PG might not only affect the size of the sample but also its taxonomic composition. Höfer et al. (2015) reported that pitfall traps deployed with PG captured significantly more spider species, but were not selectively attractive for par-

ticular taxa. Nor was the pattern consistent across all sampling sites. Reliable reference data for PG capture rates still seem to be too small to make valid statements, but the community should be aware that such a taxonomic bias could exist. Additionally, mammals can be attracted by the use of PG, disturbing pitfall traps and biasing sampling designs (e.g. Aristophanous 2010 and own observations).

Application of propylene glycol as a preservative

Propylene glycol was only occasionally used as a medium-term preservative or storage medium. After PG fixation, most samples were stored in ≥95% ethanol until DNA extraction or PG-fixed samples directly analysed within a few days when retrieved from the field. Nevertheless, Nakamura et al. (2020) highlighted the potential use of PG as a chemical agent that can be applied from trapping to storage and for various taxonomic groups, so that hands-on times can be shortened as well as labour and equipment costs reduced (i.e. no specimen picking, circumventing transfer into another solution and container). The authors compared the COI-amplification rates of dipterans, hymenopterans and coleopterans preserved in 98% PG and 99.5% ethanol over a period of 2 weeks to >6 months at room temperature, concluding that DNA might be more long-term stable in PG than in ethanol. On the contrary, Patrick et al. (2016) tested various storage agents (including 99.5% PG and 97–100% ethanol) applicable in remote areas on three dipteran species under different temperatures. Although the experiment ended after 15 days, their results suggest that keeping PG fixed samples as cold as possible (e.g. packed in ice-filled boxes from hotel bars) is important to ensure short- to mid-term DNA integrity.

If we adopt the results to common practices of sample storage, it tells us that PG-preserved samples should preferably be stored cool and dark just like ethanol-preserved samples. Yet, DNA quality and quantity of long-term stored, chilled PG-preserved samples should be investigated in further detail.

Application of propylene glycol in HTS studies

Sufficiently high DNA quantities and DNA qualities are prerequisites in HTS studies. Lienhard and Schäffer (2019) evaluated DNA quality and quantity of ethanol- and PG-preserved oribatid mite species (<1 mm, preserved for several weeks), originating from seven DNA isolation methods suitable for high-throughput DNA sequencing. Although some study parameters had a significant effect on DNA quantity and quality, results for specimens preserved in PG or absolute ethanol generally suggest a high comparability. Similarly, Carter (2003) investigated the molecular weight spectra of double-stranded DNA (dsDNA) for specimens of the Rough Woodlouse *Porcellio scaber* preserved in PG, ethanol, ethyl acetate and 2-ethoxy ethanol for 12 months at room temperature. Ethanol- and cryo-preserved specimens provided the best quality and highest concentration of high molecular weight dsDNA for the investigated time period (with a remarkable drop after 24 months for ethanol). Yet, DNA quality (in terms of degradation) and DNA quantity (in terms of concentration) of specimens stored in PG were decreased, especially for longer fragments (>5 kbp).

However, specimens were stored at room temperature, which showed a strong degradation effect in Patrick et al. (2016) compared to samples which were kept cool.

Systematic studies analysing the impact of PG fixation and preservation in the context of HTS are widely lacking. Still, first case studies indicate a high applicability of PG fixation for short read amplicon sequencing (metabarcoding; Bowser et al. 2017, 2019; Lefort et al. 2017; Jusino et al. 2019; Liu et al. 2020; Robinson et al. 2020). In particular Robinson et al. (2020) tested the effect of PG antifreeze fixation of homogenised mock communities and benthic bulk samples. Their COI metabarcoding results indicate a generally high comparability of communities from ethanol and PG fixed samples. The latter even produced a higher proportion of arthropod reads and a higher richness of exact sequence variants (ESVs) when compared to ethanol samples.

Besides studies on short read amplicon sequencing, the applicability of PG was shown for short read reduced representation methods (RAD-sequencing; Perry et al. 2017; Angelella et al. 2019; Ballare et al. 2020). In particular, Ballare et al. (2020) conducted a comparative setting testing the impact of different field sample methods for SNP detection in wild bees, including PG-filled Vane traps. While a suitable DNA concentration and high locus depth were found for PG-fixed specimens, the number of loci recovered was comparatively low. Still, the two targeted wild bee species on average possessed more than 10,000 loci with a mean locus depth of >70. One potential cause for the lower than average mean number of polymorphic loci might be an incomplete PG evaporation, leading to an insufficient ethanol preservation prior to pinning. Alternatively, PG-stored samples were kept at room temperature, whereas other samples which showed higher numbers of polymorphic loci were stored at -20 °C.

To the best of our knowledge, no study used PG-fixed or -preserved specimens for invertebrate genome sequencing. Our assumption, however, would be that if samples are fixed and stored under optimal conditions, genome sequencing based on short read lengths should be possible. However, how ultra-long sequencing (e.g. Nanopore) will be affected by potential DNA degradation effects of PG remains unclear and should be explicitly addressed.

Integrative use cases of propylene glycol-conserved invertebrate samples

Besides the possibility to perform DNA-based analyses (e.g. microsatellite fingerprinting, DNA barcoding, metabarcoding and RAD-seq) directly on the invertebrates trapped or stored in PG, the samples seem suitable for a variety of research designs. Firstly, it has to be highlighted that PG-conserved specimens demonstrate a reduced shrinkage effect and specimens often remain appropriately conserved for morphological examina-

tions (Thomas 2008). More so, PG can be even added to ethanol-preserved specimens to retain flexibility and to reduce shrinkage effects without compromising DNA integrity and storage conditions (Boaze and Waller 1992; Carter 2003), e.g. for improving morphological determinability and dissection conditions (Karanovic et al. 2012, 2016; Martin 2016; Perina et al. 2018; Herrera Russert 2019). This 'relaxation' property of PG was exploited by several studies examining DNA markers and morphology of the very same specimens (Villacorta et al. 2008; Krosch et al. 2013; Boontop et al. 2017b; Gregoire Taillefer and Wheeler 2018; Grando et al. 2018). Furthermore, PG-conserved invertebrate samples were highly suitable for related integrative study designs, which are becoming increasingly popular in modern biodiversity studies. As such, Hu et al. (2017) investigated insect-associated microbiota by 16S rRNA amplicon sequencing of ants, Tremblay et al. (2018, 2019) screened phytopathogenic fungal propagules captured in insect traps via metagenomics, and Lynggaard et al. (2019) analysed bulk arthropod Malaise trap samples to detect vertebrates via 12S and 16S DNA metabarcoding. Molecular gut content analyses of PG-fixed invertebrate samples were e.g. performed for mosquitos (Muturi et al. 2018), fruit flies (Diepenbrock et al. 2018), carrion-feeding flies (Bagnall 2016) and diverse predatory groups (Murtiningsih 2014; Mabin et al. 2020). Endoparasites were investigated by e.g. Sokolova et al. (2010; microsporidians in booklice), Looney et al. (2012; horsehair worms in carabids and crickets), Barratt et al. (2012; braconid parasitoids in weevils) and Hartshorn et al. (2016; nematodes in wood wasps). Nie et al. (2011) detected the Potato virus Y from PG-fixed aphids via reverse transcription PCR. Finally, an addition of PG to pheromone/food traps might extend the lifetime of kairomones (Faleiro et al. 2016). Propylene glycol fixation of samples thus seems to be promising for a variety of integrative study designs.

Conclusions

There is currently only limited scientific literature on the use of PG for DNA-based analyses of invertebrates available, and even less so in the context of HTS. However, the investigated studies indicate that PG can be a versatile and worthwhile alternative for sample fixation (and potentially preservation) of various organism groups and in a range of methodological setups. Yet, generally valid statements about fixatives and preservatives are difficult to make (Nagy 2010; Short et al. 2018), and can be biased by comparing agents with contrasting water conditions affecting DNA integrity (see Nakamura et al. 2020), varying field vs. laboratory humidity conditions (due to the hygroscopic nature of PG) and storage temperatures or by confusing chemicals (e.g. Vaudo et al. 2018).

Future studies which plan the application of PG should critically scrutinize their trapping, specimen and storage conditions. For how many days are traps deployed? How will humidity, precipitation, UV exposure and temperature conditions in the sampling area affect the fixative? Do the targeted organisms allow for an easy tissue penetration by the fixative (e.g. soft-bodied vs. sclerotinised specimens)? Can PG reduce hands-on times and yet overall costs (e.g. no sample transfer for shipping; no evaporation prior to DNA isolation) (see e.g. Robinson et al. 2020)? In many cases, and in particular for new large-scale DNA-based monitoring programmes, one should not simply go by tradition but perform environment- and target group-specific tests and cost calculations before deciding upon the most suitable fixative.

Acknowledgements

The work is part of the EU COST Action CA15219 ("DNAqua-Net"). It was financially supported by the German Research Foundation (grant WE 6055/1-1) and the Bauer and Stemmler foundations program "FORSCHUNGSGEIST! Next Generation Sequencing in der Oekosystemforschung".

References

Angelella GM, Michel AP, Kaplan I (2019) Using host-associated differentiation to track source population and dispersal distance among insect vectors of plant pathogens. Evolutionary Applications 12(4): 692–704. https://doi.org/10.1111/eva.12733

Aristophanous M (2010) Does your preservative preserve? A comparison of the efficacy of some pitfall trap solutions in preserving the internal reproductive organs of dung beetles. ZooKeys 34: 1–16. https://doi.org/10.3897/zookeys.34.215

Aylagas E, Borja Á, Irigoien X, Rodríguez-Ezpeleta N (2016) Benchmarking DNA metabarcoding for biodiversity-based monitoring and assessment. Frontiers in Marine Science 3: e96. https://doi.org/10.3389/fmars.2016.00096

Bagnall AJ (2016) The use of iDNA in detecting elusive mammals: a case study on the Indonesian Island of Seram. MSc thesis, Clinical Veterinary Sciences, The University of Edinburgh, Edinburgh, 91 pp.

Bank HL, Brockbank KG (1987) Basic principles of cryobiology. Journal of cardiac surgery 2(1S): 137–143. https://doi.org/10.1111/jocs.1987.2.1s.137

Barratt BIP, Oberprieler RG, Barton DM, Mouna M, Stevens M, Alonso-Zarazaga MA, Vink CJ, Ferguson CM (2012) Could research in the native range, and non-target host range in Australia, have helped predict host range of the parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a biological control agent introduced for *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) in New Zealand? BioControl 57(6): 735–750. https://doi.org/10.1007/s10526-012-9453-3

Boaze NA, Waller RR (1992) The effect of propylene glycol on ethanol concentrations determined by density measurement. Collection forum 10(2): 41–49.

Boontop Y (2017) Natural variation and biogeography of the melon fruit fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae), in Southeast-Asia and the west-pacific. PhD thesis. Queensland University of Technology, Brisbane, 377 pp.

Boontop Y, Kumaran N, Schutze MK, Clarke AR, Cameron SL, Krosch MN (2017a) Population structure in *Zeugodacus cucurbitae* (Diptera: Tephritidae) across Thailand and the Thai–Malay peninsula: natural barriers to a great disperser. Biological Journal of the Linnean Society 121(3): 540–555. https://doi.org/10.1093/biolinnean/blx009

Boontop Y, Schutze MK, Clarke AR, Cameron SL, Krosch MN (2017b) Signatures of invasion: using an integrative approach to infer the spread of melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae), across Southeast Asia and the West Pacific. Biological Invasions 19(5): 1597–1619. https://doi.org/10.1007/s10530-017-1382-8

Borges PA, Serrano AR, Amorim I, Terzopoulou S, Rigal F, Emerson B (2016) Cryptic diversity in the Azorean beetle genus *Tarphius* Erichson, 1845 (Coleoptera: Zopheridae): An integrative taxonomic approach with description of four new species. Zootaxa 4176(3): 401–449. https://doi.org/10.11646/zootaxa.4236.3.1

Bowser ML, Brassfield R, Dziergowski A, Eskelin T, Hester J, Magness DR, Stone J (2020) Towards conserving natural diversity: A biotic inventory by observations, specimens, DNA barcoding and high-throughput sequencing methods. Biodiversity Data Journal 8: e50124. https://doi.org/10.3897/BDJ.8.e50124

Bowser ML, Morton JM, Hanson JD, Magness DR, Okuly M (2017) Arthropod and oligochaete assemblages from grasslands of the southern Kenai Peninsula, Alaska. Biodiversity Data Journal 5: e10792. https://doi.org/10.3897/BDJ.5.e10792

Boyer S, Blakemore RJ, Wratten SD (2011) An integrative taxonomic approach to the identification of three new New Zealand endemic earthworm species (Acanthodrilidae, Octochaetidae: Oligochaeta). Zootaxa 2994(1): 21–32. https://doi.org/10.11646/zootaxa.2994.1.2

Boykin LM, Schutze MK, Krosch MN, Chomič A, Chapman TA, Englezou A, Armstrong KF, Clarke AR, Hailstones D, Cameron SL (2014) Multi-gene phylogenetic analysis of south-east Asian pest members of the *Bactrocera dorsalis* species complex (Diptera: Tephritidae) does not support current taxonomy. Journal of Applied Entomology 138(4): 235–253. https://doi.org/10.1111/jen.12047

Brown GR, Matthews IM (2016) A review of extensive variation in the design of pitfall traps and a proposal for a standard pitfall trap design for monitoring ground-active arthropod biodiversity. Ecology and Evolution 6(12): 3953–3964. https://doi.org/10.1002/ece3.2176

Calixto AA, Harris MK, Dean A (2007) Sampling ants with pitfall traps using either propylene glycol or water as a preservative. Southwestern Entomologist 32(2): 87–91. https://doi.org/10.3958/0147-1724-32.2.87

Carter JD (2003) The effects of preservation and conservation treatments on the DNA of museum invertebrate fluid preserved collections. MSc thesis. National Museum and Gallery of Wales, Cardiff, 136 pp.

Castalanelli MA, Baker AM, Munyard KA, Grimm M, Groth DM (2012) Molecular phylogeny supports the paraphyletic nature of the genus *Trogoderma* (Coleoptera: Dermestidae) collected in the Australasian ecozone. Bulletin of Entomological Research 102(1): 17–28. https://doi.org/10.1017/S0007485311000319

Castalanelli MA, Severtson DL, Brumley CJ, Szito A, Foottit RG, Grimm M, Munyard K, Groth DM (2010) A rapid non-destructive DNA extraction method for insects and other arthropods. Journal of Asia-Pacific Entomology 13(3): 243–248. https://doi.org/10.1016/j.aspen.2010.04.003

Chinvinijkul S, Srikachar S Kumjing P, Kimjong W, Sukamnouyporn W, Polchaimat N (2015) Inter-regional mating compatibility among *Bactrocera dorsalis* populations in Thailand (Diptera, Tephritidae). ZooKeys 540: 299–311. https://doi.org/10.3897/zookeys.540.6568

Compson ZG, McClenaghan B, Singer GAC, Fahner NA, Hajibabaei M (2020) Metabarcoding From Microbes to Mammals: Comprehensive Bioassessment on a Global Scale. Frontiers in Ecology and Evolution 8: e581835. https://doi.org/10.3389/fevo.2020.581835

Cordero RD, Sánchez-Ramírez S, Currie DC (2017) DNA barcoding of aquatic insects reveals unforeseen diversity and recurrent population divergence patterns through broad-scale sampling in northern Canada. Polar Biology 40(8): 1687–1695. https://doi.org/10.1007/s00300-016-2062-3

Coulson RN, Pinto MA, Tchakerian MD, Baum KA, Rubink WL, Johnston JS (2005) Feral honey bees in pine forest landscapes of east Texas. Forest ecology and management 215(1–3): 91–102. https://doi.org/10.1016/j.foreco.2005.05.005

Daglish GJ, Rajeswaran J, Singarayan V, Nath NS, Schlipalius DI, Ebert PR, Nayak MK (2018) Potential for using pheromone trapping and molecular screening in phosphine resistance research. Proceedings of the 12th International Working Conference on Stored Product Protection (IWCSPP), 1013–1017.

Diepenbrock LM, Lundgren JG, Sit TL, Burrack HJ (2018) Detecting Specific Resource Use by *Drosophila suzukii* (Diptera: Drosophilidae) Using Gut Content Analysis. Journal of economic entomology 111(3): 1496–1500. https://doi.org/10.1093/jee/toy077

DiGirolomo MF, Jendek E, Grebennikov VV, Nakládal O (2019) First North American record of an unnamed West Palaearctic *Agrilus* (Coleoptera: Buprestidae) infesting European beech (*Fagus sylvatica*) in New York City, USA. European Journal of Entomology 116: 244–252. https://doi.org/10.14411/eje.2019.028

Eigenbrode SD, Davis TS, Adams JR, Husebye DS, Waits LP, Hawthorne D (2016) Host-adapted aphid populations differ in their migratory patterns and capacity to colonize crops. Journal of Applied Ecology 53(5): 1382–1390. https://doi.org/10.1111/1365-2664.12693

Endo Y, Nash M, Hoffmann AA, Slatyer R, Miller AD (2014) Comparative phylogeography of alpine invertebrates indicates deep lineage diversification and historical refugia in the Australian Alps. Journal of Biogeography 42(1): 89–102. https://doi.org/10.1111/jbi.12387

Faleiro JR, Jaques JA, Carrillo D, Giblin-Davis R, Mannion CM, Peña-Rojas E, Peña JE (2016) Integrated pest management (IPM) of palm pests. Integrated Pest Management in the Tropics. New India Publishing Agency, New Delhi, 439–497.

Ferro ML, Park JS (2013) Effect of propylene glycol concentration on mid-term DNA preservation of Coleoptera. The Coleopterists Bulletin 67(4): 581–586. https://doi.org/10.1649/0010-065X-67.4.581

Fountain ED, Malumbres-Olarte J, Cruickshank RH, Paterson AM (2015) The effects of island forest restoration on open habitat specialists: the endangered weevil *Hadramphus spinipennis* Broun and its host-plant Aciphylla dieffenbachii Kirk. PeerJ 3: e749. https://doi.org/10.7717/peerj.749

Gallego D, Galián J (2008) Hierarchical structure of mitochondrial lineages of *Tomicus destruens* (Coleoptera, Scolytidae) related to environmental variables. Journal of Zoological Systematics and Evolutionary Research 46(4): 331–339. https://doi.org/10.1111/j.1439-0469.2008.00472.x

Gibson CM, Kao RH, Blevins KK, Travers PD (2012) Integrative taxonomy for continental-scale terrestrial insect observations. PLoS ONE 7(5): e37528. https://doi.org/10.1371/journal.pone.0037528

Goetze E, Jungbluth MJ (2013) Acetone preservation for zooplankton molecular studies. Journal of plankton research 35(5): 972–981. https://doi.org/10.1093/plankt/fbt035

Gómez RA (2014) On the Systematics of the North American Ground Beetle Genus Rhadine Leconte (Coleoptera: Carabidae: Platynini) with Emphasis on the Sky Island Fauna of Arizona. MSc thesis, Graduate Interdisciplinary Program in Entomology and Insect Science, The University of Arizona, 77 pp.

Gossner MM, Struwe JF, Sturm S, Max S, McCutcheon M, Weisser WW, Zytynska SE (2016) Searching for the optimal sampling solution: variation in invertebrate communities, sample condition and DNA quality. PLoS ONE 11(2): e0148247. https://doi.org/10.1371/journal.pone.0148247

Grando C, Amon ND, Clough SJ, Guo N, Wei W, Azevedo P, López-Uribe MM, Zucchi MI (2018) Two colors, one species: the case of *Melissodes nigroaenea* (Apidae: Eucerini), an important pollinator of cotton fields in Brazil. Sociobiology 65(4): 645–653. https://doi.org/10.13102/sociobiology.v65i4.3464

Greenslade P, Boyer S, Shields MW, Wratten SD (2017) First record of a possible predatory collembolan species, *Dicyrtoma fusca* (Collembola: Dicyrtomidae), in New Zealand. Austral Entomology 56(3): 332–338. https://doi.org/10.1111/aen.12240

Gregoire Taillefer A, Wheeler TA (2018) Tracking wetland community evolution using Diptera taxonomic, functional and phylogenetic structure. Insect Conservation and Diversity 11(3): 276–293. https://doi.org/10.1111/icad.12271

Gruber MAM, Burne AR, Abbott KL, Pierce RJ, Lester PJ (2013) Population decline but increased distribution of an invasive ant genotype on a Pacific atoll. Biological Invasions 15(3): 599–612. https://doi.org/10.1007/s10530-012-0312-z

Haase M, Zielske S (2015) Five new cryptic freshwater gastropod species from New Caledonia (Caenogastropoda, Truncatelloidea, Tateidae). ZooKeys 523: 63–87. https://doi.org/10.3897/zookeys.523.6066

Hartshorn JA, Fisher JR, Riggins JJ, Stephen FM (2017) Molecular identification of *Deladenus proximus* Bedding, 1974 (Tylenchida: Neotylenchidae), a parasite of Sirex nigricornis (Hymenoptera: Siricidae). Nematology 19(1): 15–20. https://doi.org/10.1163/15685411-00003029

Herrera Russert J (2019) Ecology and Conservation of *Cephalota* (*Taenidia*) *deserticoloides* (Codina, 1931) (Coleoptera, Cicindelidae). Proyecto de investigación. PhD thesis. Universidad de Murcia, Spain, 120 pp.

Hezavehei M, Sharafi M, Kouchesfahani HM, Henkel R, Agarwal A, Esmaeili V, Shahverdi A (2018) Sperm cryopreservation: a review on current molecular cryobiology and advanced approaches. Reproductive biomedicine online 37(3): 327–339. https://doi.org/10.1016/j.rbmo.2018.05.012

Höfer H, Astrin J, Holstein J, Spelda J, Meyer F, Zarte N (2015) Propylene glycol–a useful capture preservative for spiders for DNA barcoding. Arachnologische Mitteilungen 50: 30–36. https://doi.org/10.5431/aramit5005

Hoekman D, LeVan KE, Gibson C, Ball GE, Browne RA, Davidson RL, Erwin TL, Knisley CB, LaBonte JR, Lundgren J, Maddison DR, Moore W, Niemelä J, Ober KA, Pearson DL, Spence JR, Will K, Work T (2017) Design for ground beetle abundance and diversity sampling within the National Ecological Observatory Network. Ecosphere 8(4): e01744. https://doi.org/10.1002/ecs2.1744

Hohbein RR, Conway CJ (2018) Pitfall traps: A review of methods for estimating arthropod abundance. Wildlife Society Bulletin 42(4): 597–606. https://doi.org/10.1002/wsb.928

Holderegger R, Stapfer A, Schmidt BR, Grünig C, Meier R, Csencsics D, Gassner M (2019) Werkzeugkasten Naturschutzgenetik: eDNA Amphibien und Verbund. WSL Berichte 81: 1–56.

Horn ME (2010) A comparison of pollinator biodiversity between green spaces, industrial areas and residential land-use zones in urban, southern Ontario. Master thesis. Faculty of Graduate Studies of The University of Guelph, Guelph, 95 pp.

Hu YI, Holway DA, Łukasik P, Chau L, Kay AD, LeBrun EG, Miller KA, Sanders JG, Suarez AV, Russell JA (2017) By their own devices: invasive Argentine ants have shifted diet without clear aid from symbiotic microbes. Molecular Ecology 26(6): 1608–1630. https://doi.org/10.1111/mec.13991

Ide T, Kanzaki N, Masuya H, Okabe K (2018) Application of the LAMP assay for the detection of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae), from captures of pan traps. Applied Entomology and Zoology 53(2): 275–279. https://doi.org/10.1007/s13355-018-0546-z

Jusino MA, Banik MT, Palmer JM, Wray AK, Xiao L, Pelton E, Barber JR, Kawahara AY, Gratton C, Peery MZ, Lindner DL (2019) An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. Molecular Ecology Resources 19(1): 176–190. https://doi.org/10.1111/1755-0998.12951

Karanovic T, Cho JL, Lee W (2012) Redefinition of the parastenocaridid genus *Proserpinicaris* (Copepoda: Harpacticoida), with description of three new species from Korea. Journal of Natural History 46(25–26): 1573–1613. https://doi.org/10.1080/00222933.2012.681316

Karanovic T, Djurakic M, Eberhard SM (2016) Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. Systematic Biology 65(2): 304–327. https://doi.org/10.1093/sysbio/syv088

Knee WH (2012) Host specificity and species boundaries of beetle-associated mites. PhD thesis, Carleton University. Ottawa, Ontario, 212 pp.

Knee W, Beaulieu F, Skevington JH, Kelso S, Cognato AI, Forbes MR (2012) Species boundaries and host range of tortoise mites (Uropodoidea) phoretic on bark beetles (Scolytinae), using morphometric and molecular markers. PLoS ONE 7(10): e47243. https://doi.org/10.1371/journal.pone.0047243

Kotze DJ, Brandmayr P, Casale A, Dauffy-Richard E, Dekoninck W, Koivula MJ, Lövei GL, Mossakowski D, Noordijk J, Paarmann W, Pizzolotto R, Saska P, Schwerk A, Serrano J, Szyszko J, Taboada A, Turin H, Venn S, Vermeulen R, Zetto T (2011) Forty years of carabid beetle research in Europe-from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys 100: 55–148. https://doi.org/10.3897/zookeys.100.1523

Krosch MN, Schutze MK, Armstrong KF, Boontop Y, Boykin LM, Chapman TA, Englezou A, Cameron SL, Clarke AR (2013) Piecing together an integrative taxonomic puzzle: microsatellite, wing shape and aedeagus length analyses of *Bactrocera dorsalis* sl (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. Systematic Entomology 38(1): 2–13. https://doi.org/10.1111/j.1365-3113.2012.00643.x

Krosch MN, Schutze MK, Newman J, Strutt F, Bryant LM, McMahon J, Clarke AR (2019) In the footsteps of Wallace: population structure in the breadfruit fruit fly, *Bactrocera umbrosa* (F.) (Diptera: Tephritidae), suggests disjunction across the Indo-Australian Archipelago. Australian Entomology 58(3): 602–613. https://doi.org/10.1111/aen.12375

Krosch MN, Strutt F, Blacket MJ, Batovska J, Starkie M, Clarke AR, Cameron SL, Schutze MK (2020) Development of internal COI primers to improve and extend barcoding of fruit flies (Diptera: Tephritidae: Dacini). Insect Science 27(1): 143–158. https://doi.org/10.1111/1744-7917.12612

Lagos-Kutz D, Voegtlin DJ, Onstad D, Hogg D, Ragsdale D, Tilmon K, Hodgson E, Difonzo C, Groves R, Krupke C, Laforest J, Seiter NJ, Duerr E, Bradford B, Hartman GL (2020) The Soybean Aphid Suction Trap Network: Sampling the Aerobiological "Soup". American Entomologist 66(1): 48–55. https://doi.org/10.1093/ae/tmaa009

Landi L, Braccini CL, Knížek M, Pereyra VA, Marvaldi AE (2019) A newly detected exotic ambrosia beetle in Argentina: *Euwallacea interjectus* (Coleoptera: Curculionidae: Scolytinae). Florida Entomologist 102(1): 240–242. https://doi.org/10.1653/024.102.0141

Langer SV, Kyle CJ, Beresford DV (2017) Using frons width to differentiate blow fly species (Diptera: Calliphoridae) *Phormia regina* (Meigen) and *Protophormia terraenovae* (Robineau-Desvoidy). Journal of Forensic Sciences 62(2): 473–475. https://doi.org/10.1111/1556-4029.13281

Leblanc L, Fay H, Sengebau F, San Jose M, Rubinoff D, Pereira R (2015) A survey of fruit flies (Diptera: Tephritidae: Dacinae) and their Opiine parasitoids (Hymenoptera: Braconidae) in Palau. Proceedings of the Hawaiian Entomological Society 47: 55–66.

Leese F, Bouchez A, Abarenkov K, Altermatt F, Borja Á, Bruce K, Ekrem T, Čiampor Jr F, Čiamporová-Zaťovičová Z, Costa FO, Duarte S, Elbrecht V, Fontaneto D, Franc A, Geiger MF, Hering D, Kahlert M, Stroil BK, Kelly M, Keskin E, Liska I, Mergen P, Meissner K, Pawlowski J, Penev L, Reyjol Y, Rotter A, Steinke D, Van der Wal B, Vitecek S, Zimmermann J, Weigand AM (2018) Chapter Two – Why We Need Sustainable Networks Bridging Countries, Disciplines, Cultures and Generations for Aquatic Biomonitoring 2.0: A Perspective Derived From the DNAqua-Net COST Action. In: Bohan DA, Dumbrell AJ, Woodward G, Jackson M (Eds) Advances in Ecological Research, Next Generation Biomonitoring: Part 1. Academic Press, 63–99. https://doi.org/10.1016/bs.aecr.2018.01.001

Lefort MC, Wratten S, Cusumano A, Varennes YD, Boyer S (2017) Disentangling higher trophic level interactions in the cabbage aphid food web using high-throughput DNA sequencing. Metabarcoding and Metagenomics 1: e13709. https://doi.org/10.3897/mbmg.1.13709

Lienhard A, Schäffer S (2019) Extracting the invisible: obtaining high quality DNA is a challenging task in small arthropods. PeerJ 7: e6753. https://doi.org/10.7717/peerj.6753

Liu YH (2016) Do Corridors Increase Gene Flow for Invasive Species? Master thesis. North Carolina State University, Raleigh, 42 pp.

Liu M, Baker SC, Burridge CP, Jordan GJ, Clarke LJ (2020) DNA metabarcoding captures subtle differences in forest beetle communities following disturbance. Restoration Ecology 28(6): 1475–1484. https://doi.org/10.1111/rec.13236

Liu M, Clarke LJ, Baker SC, Jordan GJ, Burridge CP (2020) A practical guide to DNA metabarcoding for entomological ecologists. Ecological Entomology 45(3): 373–385. https://doi.org/10.1111/een.12831

Looney C, Hanelt B, Zack RS (2012) New records of nematomorph parasites (Nematomorpha: Gordiida) of ground beetles (Coleoptera: Carabidae) and camel crickets (Orthoptera: Rhaphidophoridae) in Washington State. Journal of Parasitology 98(3): 554–559. https://doi.org/10.1645/GE-2929.1

López H, Oromí P (2010) A pitfall trap for sampling the mesovoid shallow substratum (MSS) fauna. Speleobiology Notes 2: 7–11.

Lynggaard C, Nielsen M, Santos-Bay L, Gastauer M, Oliveira G, Bohmann K (2019) Vertebrate diversity revealed by metabarcoding of bulk arthropod samples from tropical forests. Environmental DNA 1(4): 329–341. https://doi.org/10.1002/edn3.34

Mabin MD, Welty C, Gardiner MM (2020) Predator richness predicts pest suppression within organic and conventional summer squash (*Cucurbita pepo* L. Cucurbitales: Cucurbitaceae). Agriculture, Ecosystems & Environment 287: e106689. https://doi.org/10.1016/j.agee.2019.106689

Mahon MB, Campbell KU, Crist TO (2017) Effectiveness of Winkler litter extraction and pitfall traps in sampling ant communities and functional groups in a temperate forest. Environmental Entomology 46(3): 470–479. https://doi.org/10.1093/ee/nvx061

Malumbres Olarte J (2010) Spider diversity and ecology in native tussock grasslands of the South Island, New Zealand. PhD thesis. Lincoln University, Christchurch, 242 pp.

Martin JW (2016) Collecting and processing crustaceans: an introduction. Journal of Crustacean Biology 36(3): 393–395. https://doi.org/10.1163/1937240X-00002436

Matos-Maraví P, Ritter CD, Barnes CJ, Nielsen M, Olsson U, Wahlberg N, Marquina D, Sääksjärvi I, Antonelli A (2019) Biodiversity seen through the perspective of insects: 10 simple rules on methodological choices and experimental design for genomic studies. PeerJ 7: e6727. https://doi.org/10.7717/peerj.6727

McCravy KW, Willand JE (2007) Effects of pitfall trap preservative on collections of carabid beetles (Coleoptera: Carabidae). The Great Lakes Entomologist 40(3): 154–165.

Mitchell A, Moeseneder CH, Hutchinson PM (2020) Hiding in plain sight: DNA barcoding suggests cryptic species in all 'well-known' Australian flower beetles (Scarabaeidae: Cetoniinae). PeerJ 8: e9348. https://doi.org/10.7717/peerj.9348

Moricca S, Bracalini M, Benigno A, Ghelardini L, Furtado EL, Marino CL, Panzavolta T (2020) Observations on the non-native Thousand Cankers Disease of Walnut in Europe's southernmost outbreak. Global Ecology and Conservation: e01159. https://doi.org/10.1016/j.gecco.2020.e01159

Murtiningsih R (2014) Predation of cabbage pests: a combined ecological and molecular approach. PhD thesis. University of Queensland, School of Biological Sciences, Brisbane, 176 pp.

Muturi EJ, Lagos-Kutz D, Dunlap C, Ramirez JL, Rooney AP, Hartman GL, Fields CJ, Rendon G, Kim CH (2018) Mosquito microbiota cluster by host sampling location. Parasites & Vectors 11(1): e468. https://doi.org/10.1186/s13071-018-3036-9

Nakahama N, Isagi Y, Ito M (2019) Methods for retaining well-preserved DNA with dried specimens of insects. European Journal of Entomology 116: 486–491. https://doi.org/10.14411/eje.2019.050

Nakamura S, Tamura S, Taki H, Shoda-Kagaya E (2020) Propylene glycol: a promising preservative for insects, comparable to ethanol, from trapping to DNA analysis. Entomologia Experimentalis et Applicata 168(2): 158–165. https://doi.org/10.1111/eea.12876

Nagy ZT (2010) A hands-on overview of tissue preservation methods for molecular genetic analyses. Organisms Diversity & Evolution 10(1): 91–105. https://doi.org/10.1007/s13127-010-0012-4

Nie X, Pelletier Y, Mason N, Dilworth A, Giguère MA (2011) Aphids preserved in propylene glycol can be used for reverse transcription-polymerase chain reaction detection of Potato virus Y. Journal of virological methods 175(2): 224–227. https://doi.org/10.1016/j.jviromet.2011.05.019

Patrick HJH, Chomič A, Armstrong KF (2016) Cooled propylene glycol as a pragmatic choice for preservation of DNA from remote field-collected Diptera for next-generation sequence analysis. Journal of Economic Entomology 109(3): 1469–1473. https://doi.org/10.1093/jee/tow047

Pelletier Y, Nie X, Giguère MA, Nanayakkara U, Maw E, Foottit R (2012) A new approach for the identification of aphid vectors (Hemiptera: Aphididae) of potato virus Y. Journal of Economic Entomology 105(6): 1909–1914. https://doi.org/10.1603/EC12085

Perina G, Camacho AI, Huey J, Horwitz P, Koenders A (2018) Understanding subterranean variability: the first genus of Bathynellidae (Bathynellacea, Crustacea) from Western Australia described through a morphological and multigene approach. Invertebrate Systematics 32(2): 423–447. https://doi.org/10.1071/IS17004

Perry KD, Pederson SM, Baxter SW (2017) Genome-wide SNP discovery in field and laboratory colonies of Australian *Plutella* species. BioRxiv: e141606. https://doi.org/10.1101/141606

Piasecki J, Waligora M, Dranseika V (2018) Google search as an additional source in systematic reviews. Science and engineering ethics 24(2): 809–810. https://doi.org/10.1007/s11948-017-0010-4

Postlethwaite JV (2016) Biogeography of Chilean bees in the Atacama Desert: with a focus on the boundary between summer and winter rainfall regions. Master thesis, Faculty of Graduate Studies. York University, Toronto, 95 pp.

Ramírez MJ, Grismado CJ, Ubick D, Ovtsharenko V, Cushing PE, Platnick NI, Wheeler WC, Prendini L, Crowley LM, Horner NV (2019) Myrmecicultoridae, a new family of myrmecophilic spiders from the Chihuahuan Desert (Araneae: Entelegynae). American Museum Novitates 2019(3930): 1–24. https://doi.org/10.1206/3930.1

Renaud AK (2012) Biodiversity of the Muscidae (Diptera) from Churchill, Manitoba, Canada, with Taxonomic Issues Revealed or Resolved by DNA Barcoding. Department of Entomology, University of Manitoba, Winnipeg, Manitoba, 233 pp.

Renaud AK, Savage J, Roughley RE (2012) Muscidae (Diptera) diversity in Churchill, Canada, between two time periods: evidence for limited changes since the Canadian Northern Insect Survey. The Canadian Entomologist 144(1): 29–51. https://doi.org/10.4039/tce.2012.6

Robideau GP, Foottit RG, Humble LM, Noseworthy MK, Wu T, Bilodeau GJ (2016) Real-time PCR identification of the ambrosia beetles, *Trypodendron domesticum* (L.) and *Trypodendron lineatum* (Olivier)(Coleoptera: Scolytidae). Journal of Applied Entomology 140(4): 299–307. https://doi.org/10.1111/jen.12254

Robinson CV, Porter TM, Wright MT, Hajibabaei M (2020) Propylene glycol-based antifreeze as an effective preservative for DNA metabarcoding of benthic arthropods. bioRxiv. https://doi.org/10.1101/2020.02.28.970475

Rubink WL, Murray KD, Baum KA, Pinto MA (2003) Long term preservation of DNA from honey bees (*Apis mellifera*) collected in aerial pitfall traps. Texas Journal of Science: 159–168.

Rugman-Jones PF, Seybold SJ, Graves AD, Stouthamer R (2015) Phylogeography of the walnut twig beetle, *Pityophthorus juglandis*, the vector of thousand cankers disease in North American walnut trees. PLoS ONE 10(2): e0118264. https://doi.org/10.1371/journal.pone.0118264

Sánchez García FJ (2015) Phylogeography, genomics and biosemiotics of bark beetles (Coleoptera: Scolytinae) = Filogeografía, genómica y biosemiótica de escarabajos de corteza (Coleoptera: Scolytinae). Universidad de Murcia, Faculty of Biology, Murcia, 206 pp.

Sánchez García FJ, Galián J, Gallego D (2015) Distribution of *Tomicus destruens* (Coleoptera: Scolytinae) mitochondrial lineages: phylogeographic insights and niche modelling. Organisms Diversity & Evolution 15(1): 101–113. https://doi.org/10.1007/s13127-014-0186-2

Schmidt MH, Clough Y, Schulz W, Westphalen A, Tscharntke T (2006) Capture efficiency and preservation attributes of different fluids in pitfall traps. The Journal of Arachnology 34(1): 159–162. https://doi.org/10.1636/T04-95.1

Schutze MK, Krosch MN, Armstrong KF, Chapman TA, Englezou A, Chomič A, Cameron SL, Hailstones D, Clarke AR (2012) Population structure of *Bactrocera dorsalis* ss, *B. papayae* and *B. philippinensis* (Diptera: Tephritidae) in southeast Asia: evidence for a single species hypothesis using mitochondrial DNA and wing-shape data. BMC Evolutionary Biology 12(1): e130. https://doi.org/10.1186/1471-2148-12-130

Scott IAW, Workman PJ, Drayton GM, Burnip GM (2007) First record of *Bemisia tabaci* biotype Q in New Zealand. New Zealand Plant Protection 60: 264–270. https://doi.org/10.30843/nzpp.2007.60.4601

Seybold SJ, Dallara PL, Hishinuma SM, Flint ML (2013) Detecting and identifying the walnut twig beetle: Monitoring guidelines for the invasive vector of thousand cankers disease of walnut, University of California Agriculture and Natural Resources, Statewide Integrated Pest Management Program, Oakland, 13 pp. [March 13, 2013] http://www.ipm.ucdavis.edu/PMG/menu.thousandcankers.html [accessed 3 August 2020]

Shoda-Kagaya E, Saito S, Okada M, Nozaki A, Nunokawa K, Tsuda Y (2010) Genetic structure of the oak wilt vector beetle *Platypus quercivorus*: inferences toward the process of damaged area expansion. BMC Ecology 10(1): e21. https://doi.org/10.1186/1472-6785-10-21

Short AEZ, Dikow T, Moreau CS (2018) Entomological collections in the age of big data. Annual Review of Entomology 63: 513–530. https://doi.org/10.1146/annurev-ento-031616-035536

Sikes DS, Stockbridge J (2013) Description of *Caurinus tlagu*, new species, from Prince of Wales Island, Alaska (Mecoptera, Boreidae, Caurininae). ZooKeys 316: 35–53. https://doi.org/10.3897/zookeys.316.5400

Sim K (2013) Genetic analysis of *Pardosa* Wolf Spiders (Araneae: Lycosidae) across the Northern Nearctic. Master thesis, McGill University, Department of Natural Resource Sciences, Montreal, 98 pp.

Skvarla MJ, Larson JL, Dowling APG (2014) Pitfalls and preservatives: a review. The Journal of the Entomological Society of Ontario 145: 15–43.

Smith SM, Cognato AI (2015) *Ambrosiophilus peregrinus* Smith and Cognato, new species (Coleoptera: Curculionidae: Scolytinae), an exotic ambrosia beetle discovered in Georgia, USA. The Coleopterists Bulletin 69(2): 213–220. https://doi.org/10.1649/0010-065X-69.2.213

Sokolova YY, Sokolov IM, Carlton CE (2010) New microsporidia parasitizing bark lice (Insecta: Psocoptera). Journal of invertebrate pathology 104(3): 186–194. https://doi.org/10.1016/j.jip.2010.03.008

Sonoda S, Yamashita J, Kohara Y, Izumi Y, Yoshida H, Enomoto T (2010) Population survey of spiders using mt-DNA (COI) sequences in Japanese peach orchards. Applied Entomology and Zoology 46(1): 81–86. https://doi.org/10.1007/s13355-010-0011-0

Ssymank A, Sorg M, Doczkal D, Rulik B, Merkel-Wallner G, Vischer-Leopold M (2018) Praktische Hinweise und Empfehlungen zur Anwendung von Malaisefallen für Insekten in der Biodiversitätserfassung und im Monitoring. Series Naturalis 1: 1–12.

Stein ED, White BP, Mazor RD, Miller PE, Pilgrim EM (2013) Evaluating ethanol-based sample preservation to facilitate use of DNA barcoding in routine freshwater biomonitoring programs using benthic macroinvertebrates. PLoS ONE 8(1): e51273. https://doi.org/10.1371/journal.pone.0051273

Steininger S, Storer C, Hulcr J, Lucky A (2015) Alternative preservatives of insect DNA for citizen science and other low-cost applications. Invertebrate Systematics 29(5): 468–472. https://doi.org/10.1071/IS15003

Stevens MM, Warren GN, Mo J, Schlipalius DI (2011) Maintaining DNA quality in stored-grain beetles caught in Lindgren funnel traps. Journal of Stored Products Research 47(2): 69–75. https://doi.org/10.1016/j.jspr.2010.10.002

Stockbridge JM (2013) Beetles and spiders as indicators of forest recovery on Prince of Wales Island, Alaska. PhD thesis. University of Alaska Fairbanks, Fairbanks, 148 pp.

Stoeckle BC, Dworschak K, Gossner MM, Kuehn R (2010) Influence of arthropod sampling solutions on insect genotyping reliability. Entomologia Experimentalis et Applicata 135(2): 217–223. https://doi.org/10.1111/j.1570-7458.2010.00977.x

Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E (2012) Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular ecology 21(8): 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Taillefer AG, Wheeler TA (2019) Latitudinal patterns in phylogenetic and functional diversity of Diptera in temperate bogs. The Canadian Entomologist 151(2): 187–208. https://doi.org/10.4039/tce.2019.5

Thomas DB (2008) Nontoxic antifreeze for insect traps. Entomological News 119(4): 361–365. https://doi.org/10.3157/0013-872X-119.4.361

Tremblay ÉD, Duceppe MO, Bérubé JA, Kimoto T, Lemieux C, Bilodeau GJ (2018) Screening for exotic forest pathogens to increase survey capacity using metagenomics. Phytopathology 108(12): 1509–1521. https://doi.org/10.1094/PHYTO-02-18-0028-R

Tremblay ÉD, Kimoto T, Bérubé JA, Bilodeau GJ (2019) High-Throughput Sequencing to Investigate Phytopathogenic Fungal Propagules Caught in Baited Insect Traps. Journal of Fungi 5(1): e15. https://doi.org/10.3390/jof5010015

Ulyshen MD, Zachos LG, Stireman III JO, Sheehan TN, Garrick RC (2017) Insights into the ecology, genetics and distribution of *Lucanus elaphus* Fabricius (Coleoptera: Lucanidae), North America's giant stag beetle. Insect Conservation and Diversity 10(4): 331–340. https://doi.org/10.1111/icad.12229

Vaudo AD, Fritz ML, López-Uribe MM (2018) Opening the door to the past: Accessing phylogenetic, pathogen, and population data from museum curated bees. Insect Systematics and Diversity 2(5): 1–4. https://doi.org/10.1093/isd/ixy014

Vélez S, Mesibov R, Giribet G (2012) Biogeography in a continental island: population structure of the relict endemic centipede *Craterostigmus tasmanianus* (Chilopoda, Craterostigmomorpha) in Tasmania using 16S rRNA and COI. Journal of Heredity 103(1): 80–91. https://doi.org/10.1093/jhered/esr110

Villacorta C, Jaume D, Oromí P, Juan C (2008) Under the volcano: phylogeography and evolution of the cave-dwelling *Palmorchestia hypogaea* (Amphipoda, Crustacea) at La Palma (Canary Islands). BMC Biology 6(1): 1–7. https://doi.org/10.1186/1741-7007-6-7

Vink CJ, Thomas SM, Paquin P, Hayashi CY, Hedin M (2005) The effects of preservatives and temperatures on arachnid DNA. Invertebrate Systematics 19(2): 99–104. https://doi.org/10.1071/IS04039

Weeks Jr RD, McIntyre NE (1997) A comparison of live versus kill pitfall trapping techniques using various killing agents. Entomologia Experimentalis et Applicata 82(3): 267–273. https://doi.org/10.1046/j.1570-7458.1997.00140.x

Wiseman BH, Fountain ED, Bowie MH, He S, Cruickshank RH (2016) Vivid molecular divergence over volcanic remnants: the phylogeography of *Megadromus guerinii* on Banks Peninsula, New Zealand. New Zealand Journal of Zoology 43(3): 246–257. https://doi.org/10.1080/03014223.2016.1167093

Supplementary material 1

Overview of DNA-based studies of invertebrates applying propylene glycol, sorted by year and taxonomic group

Author: Alexander Weigand

Data type: study counts

Explanation note: Overview of DNA-based studies of invertebrates applying propylene glycol, sorted by year and taxonomic group.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mbmg.5.57278.suppl1